

CLAIMS

1. In a method for increasing the efficiency of gene transfer into target cells with a retrovirus, wherein the improvement comprises carrying out the transduction by infecting the target cells with the retrovirus in the presence of a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

2. A method according to claim 1, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

3. A method according to claim 1, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

4. A method according to claim 3, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

5. A method according to claim 4, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

6. A method according to claim 5, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

5 7. A method according to claim 4, wherein the ligand is erythropoietin.

8. A method according to any one of claims 1 to 7, wherein the functional materials are immobilized.

9. A culture medium for target cells to be used for gene transfer into the target cells with a retrovirus which comprises a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

10 10. A culture medium according to claim 9, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

11. A culture medium according to claim 9, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

12. A culture medium according to claim 11, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

13. A culture medium according to claim 12, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

5 14. A culture medium according to claim 13, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

15. A culture medium according to claim 12, wherein the ligand is erythropoietin.

10 16. A culture medium according to any one of claims 9 to 15, wherein the functional materials are immobilized.

15 17. A method for localization of a retrovirus which comprises incubating a culture medium containing the retrovirus contacted with a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

20 18. A method for localization according to claim 17, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

25 19. A method for localization according to claim 17, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

20. A method for localization according to claim 19, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

5 21. A method for localization according to claim 20, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

10 22. A method for localization according to claim 21, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

23. A method for localization according to claim 20, wherein the ligand is erythropoietin.

15 24. A method for localization according to any one of claims 17 to 23, wherein the functional materials are immobilized.

25. A kit for carrying out retrovirus-mediated gene transfer into target cells, which comprises:

20 (a) an effective amount of a functional material having retrovirus binding domain and/or an effective amount of another functional material having target cell binding domain;

(b) an artificial substrate for incubating the retrovirus and the target cells; and

25 (c) a target cell growth factor for pre-stimulating the target cells.

5 26. A kit according to claim 25, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

27. A kit according to claim 25, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

10 28. A kit according to claim 27, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

29. A kit according to claim 27, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

15 30. A kit according to claim 29, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

31. A kit according to claim 28, wherein the ligand is erythropoietin.

20 32. A kit according to any one of claims 25 to 31, wherein the functional materials are immobilized.

33. A method for localization of a retrovirus comprising incubating a culture medium containing the retrovirus contacted with an effective amount of a functional

material having a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine.

34. A method for localization according to claim 33, wherein the functional material is immobilized.

5 35. In a method for increasing the efficiency of gene transfer into target cells with a retrovirus, wherein the improvement comprises carrying out the transduction by infecting the target cells with the retrovirus in the presence of an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

10 36. A method according to claim 35, wherein the target cell binding domain is a ligand which specifically binds to the target cells.

15 37. A method according to claim 36, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

20 38. A method according to claim 37, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

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functional equivalent of the fragment

44. A method according to claim 43, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

45. A method according to claim 35, wherein the

45. A method according to claim 35, wherein the functional material is a polypeptide having an amino acid

sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

46. A method according to any one of claims 35 to 45, wherein the functional material is immobilized.

5 47. A method according to any one of claims 35 to 45, wherein the functional material is used without immobilization.

10 48. A culture medium for target cells to be used for gene transfer into the target cells with a retrovirus which comprises an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

15 49. A culture medium according to claim 48, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

20 50. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

25 51. A culture medium according to claim 48, wherein the collagen is selected from a fragment having insulin



binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

52. A culture medium according to claim 48, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

53. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

54. A culture medium according to any one of claims 48 to 53, wherein the functional material is immobilized.

55. A method for localization of a retrovirus which comprises incubating a culture medium containing the retrovirus contacted with an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

56. A method for localization according to claim 55, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and

polypeptides containing the factor or functional equivalents of the factor.

5 57. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

10 58. A method for localization according to claim 55, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

15 59. A method for localization according to claim 58, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

60. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

20 61. A method for localization according to any one of claims 50 to 60, wherein the functional material is immobilized.

62. A kit for carrying out retrovirus-mediated gene transfer into target cells, which comprises:

(a) an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule;

(b) an artificial substrate for incubating the retrovirus and the target cells; and

(c) a target cell growth factor for pre-stimulating the target cells.

63. A kit according to claim 62, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

64. A kit according to claim 62, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

65. A kit according to claim 62, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

66. A kit according to claim 65, wherein the fragment having insulin binding domain derived from type V

collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

5 67. A kit according to claim 62, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

68. A kit according to any one of claims 62 to 67, wherein the functional material is immobilized.

10 69. A method according to claim 8 or 46, wherein the functional materials are immobilized on beads.

70. A culture medium according to claim 16 or 54, the functional materials are immobilized on beads.

15 71. A method for immobilization according to claim 24, 34 or 61, wherein the functional materials are immobilized on beads.

72. A kit according to claim 32 or 68, wherein the functional materials are immobilized on beads.

20 73. In a method for increasing the efficiency of gene transfer into target cells with a retrovirus, wherein the improvement comprises infecting the target cells with the retrovirus in the presence of an effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is immobilized  
25 on beads.

74. In a method for increasing the efficiency of gene transfer into target cells with a retrovirus, wherein the improvement comprises infecting the target cells with the retrovirus in the presence of an effective amount of a functional material selected from the group consisting of substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof which is not immobilized.

75. A method according to any one of claims 1 to 8, 35 to 47, 69, 73 and 74, wherein the target cells are cells selected from stem cells, hematopoietic cells, non-adherent low density mononuclear cells, adherent cells, bone marrow cells, hematopoietic stem cells, peripheral blood stem cells, umbilical blood cells, fetal hematopoietic stem cells, embryoplastic stem cells, embryonic cells, primordial germ cell, oocyte, oogonia, ova, spermatocyte, sperm, CD 34 + cells, C-kit + cells, multipotential hematopoietic progenitor cells, unipotential hematopoietic progenitor cells, erythrocyte precursor cells, lymphocytic precursor cells, mature blood cells, lymphocytes, B cells, T cells, fibroblasts, neuroblasts, nerve cells, endothelial cells, angio-endothelial cells, hepatic cells, myoblasts, skeletal muscle cells, smooth muscle cells, cancer cells, myeloma cells and leukemia cells.

76. A method according to any one of claims 1 to 8, 17 to 24, 33 to 47, 55 to 61, 69, 71 and 73 to 75, wherein the retrovirus includes an exogenous gene.

5 77. A method according to claim 76, wherein the retrovirus is a recombinant retroviral vector.

78. A method according to claim 76, wherein the retrovirus is a replication deficient recombinant retroviral vector.

10 79. Transformant cells obtained by a method according to any one of claims 1 to 8, 35 to 47, 69 and 73 to 78.

80. A method for cellular grafting comprising grafting the transformant cells obtained by a method according to claim 79 into a vertebrate animal.

15 81. A polypeptide represented by SEQ. ID 13 of the Sequence Listing.

82. A gene encoding the polypeptide according to claim 81.

20 83. A gene according to claim 82 which is represented by SEQ. ID No. 17 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

25 84. A polypeptide represented by SEQ. ID No. 30 of the Sequence Listing or functional equivalents thereof.

85. A gene encoding the polypeptide according to claim 84.

5 86. A gene according to claim 85 which is represented by SEQ. ID No. 33 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

10 87. A polypeptide represented by SEQ. ID No. 5 of the Sequence Listing or functional equivalents thereof.

88. A gene encoding the polypeptide according to claim 87.

15 89. A gene according to claim 88 which is represented by SEQ. ID No. 26 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

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